

MOUSE *Fkh<sup>f</sup>* cDNA SEQUENCE

1 GCTGATCCCC CTCTAGCAGT CCACTTCACC AAGGTGAGCG AGTGTCCCTG  
 51 CTCTCCCCCA CCAGACAAG CTCTGCTGGC GAAAGTGGCA GAGAGGTATT  
 101 GAGGGTGGGT GTCAGGAGCC CACCACTACA GCTGGAAACA CCCAGCCACT  
 151 CCAGCTCCCG GCAACTTCTC CTGACTCTGC CTTGAGACGA GACTTGGAAG  
 201 ACAGTCACAT CTCAGCAGCT CCTCTGCCGT TATCCAGCCT GCCTCTGACA  
 251 AGAACCCAAAT GCCCAACCCT AGGCCAGCCA AGCCTATGGC TCCTTCCTTG  
 301 GCCCTTGGCC CATCCCCAGG AGTCTTGCCA AGCTGGAAGA GTGACCCCAA  
 351 GGGCTCAGAA CTTCTAGGGA CCAGGGGCTC TGGGGGACCC TTCCAAGGTC  
 401 GGGACCTGCG AAGTGGGGCC CACACCTCTT CTTCTTGAA CCCCTGCCA  
 451 CCATCCCAGC TGCAGCTGCC TACAGTGCCC CTAGTCATGG TGGCACCGTC  
 501 TGGGGCCCGA CTAGGTCCCT CACCCACCT ACAGGCCCTT CTCCAGGACA  
 551 GACCACACTT CATGCATCAG CTCTCCACTG TGGATGCCCA TGCCAGACC  
 601 CCTGTGCTCC AAGTGCCTCC ACTGGACAAC CCAGCCATGA TCAGCCTCCC  
 651 ACCACCTTCT GCTGCCACTG GGGTCTTCTC CCTCAAGGCC CGGCCTGGCC  
 701 TGCCACCTGG GATCAATGTG GCCAGTCTGG AATGGGTGTC CAGGGAGCCA  
 751 GCTCTACTCT GCACCTTCCC ACGCTCGGGT ACACCCAGGA AAGACAGCAA  
 801 CCTTTTGGCT GCACCCCAAG GATCCTACCC ACTGCTGGCA AATGGAGTCT  
 851 GCAAGTGGCC TGGTTGTGAG AAGGTCTTCG AGGAGCCAGA AGAGTTTCTC  
 901 AAGCACTGCC AAGCAGATCA TCTCCTGGAT GAGAAAGGCA AGGCCCAGTG  
 951 CCTCCTCCAG AGAGAAGTGG TGCAGTCTCT GGAGCAGCAG CTGGAGCTGG  
 1001 AAAAGGAGAA GCTGGGAGCT ATGCAGGCCC ACCTGGCTGG GAAGATGGCG  
 1051 CTGGCCAAGG CTCCATCTGT GGCCTCAATG GACAAGAGCT CTTGCTGCAT  
 1101 CGTAGCCACC AGTACTCAGG GCAGTGTGCT CCCGGCCTGG TCTGCTCCTC  
 1151 GGGAGGCTCC AGACGGCGGC CTGTTTGCA GTCGGAGGCA CCTCTGGGGA  
 1201 AGCCATGGCA ATAGTTCCTT CCCAGAGTTC TTCCACAACA TGGACTACTT  
 1251 CAAGTACCAC AATATGCGAC CCCCTTTCAC CTATGCCACC CTTATCCGAT  
 1301 GGGCCACTCT GGAAGCCCCG GAGAGGCAGA GGACACTCAA TGAAATCTAC  
 1351 CATTGGTTTA CTCGCATGTT CGCCTACTTC AGAAACCACC CCGCCACCTG  
 1401 GAAGAATGCC ATCCGCCACA ACCTGAGCCT GCACAAGTGC TTTGTGCGAG  
 1451 TGGAGAGCGA GAAGGGAGCA GTGTGGACCG TAGATGAATT TGAGTTTCGC  
 1501 AAGAAGAGGA GCCAACGCC CAACAAGTGC TCCAATCCCT GCCCTTGACC  
 1551 TCAAAACCAA GAAAAGGTGG GCGGGGGAGG GGGCCAAAAC CATGAGACTG  
 1601 AGGCTGTGGG GGCAAGGAGG CAAGTCTTAC GTGTACCTAT GGAAACCGGG  
 1651 CGATGATGTG CCTGCTATCA GGGCCTCTGC TCCCTATCTA GCTGCCCTCC  
 1701 TAGATCATAT CATCTGCCTT ACAGCTGAGA GGGGTGCCAA TCCCAGCCTA  
 1751 GCCCCTAGTT CCAACCTAGC CCAAGATGA ACTTTCCAGT CAAAGAGCCC  
 1801 TCACAACCAG CTATACATAT CTGCCTTGGC CACTGCCAAG CAGAAAGATG  
 1851 ACAGACACCA TCCTAATATT TACTCAACCC AAACCCTAAA ACATGAAGAG  
 1901 CCTGCCTTGG TACATTCGTG AACTTTCAAA GTTAGTCATG CAGTCACACA  
 1951 TGACTGCAGT CCTACTGACT CACACCCCAA AGCACTCACC CACAACATCT  
 2001 GGAACCACGG GCACTATCAC ACATAGGTGT ATATACAGAC CCTTACACAG  
 2051 CAACAGCACT GGAACCTTCA CAATTACATC CCCCCAAACC ACACAGGCAT  
 2101 AACTGATCAT ACGCAGCCTC AAGCAATGCC CAAAATACAA GTCAGACACA  
 2151 GCTTGTGAGA

Figure 1

## MOUSE Fkh<sup>st</sup> PROTEIN SEQUENCE

1 MPNPRPAKPM APSLALGPSP GVLPSWKTAP KGSELLGTRG SGGPFQGRDL  
51 RSGAHTSSSL NPLPPSQLQL PTVPLVMVAP SGARLGPSPH LQALLQDRPH  
101 FMHQLSTVDA HAQTPVLQVR PLDNPAMISL PPPSAATGVF SLKARPGGLPP  
151 GINVASLEWV SREPALLCTF PRSGTPRKDS NLLAAPQGSY PLLANGVCKW  
201 PGCEKVFEED EEFLKHCQAD HLLDEKGKAQ CLLQREVVQS LEQQLELEKE  
251 KLGAMQAHLA GKMAKAKAPS VASMDKSSCC IVATSTQGSV LPAWSAPREA  
301 PDGGLFAVRR HLWGSHGNSS FPEFFHNMDY FKYHNMRPPF TYATLIRWAI  
351 LEAPERQRTL NEIYHWFTRM FAYFRNHPAT WKNAIRHNLS LHKCFVRVES  
401 EKGAVWTVDE FEFKRSQR PNKCSNPCP\*

Figure 2

004207 " 04E26960

# HUMAN *FKH<sup>f</sup>* cDNA Sequence

```

1  GCACACACTC ATCGAAAAA AATTGGATTA TTAGAAGAGA GAGGTCTGCG
51  GCTTCCACAC CGTACAGCGT GGTTTTCTT CTCGGTATAA AAGCAAAGTT
101 GTTTTTGATA CGTGACAGTT TCCCACAAGC CAGGCTGATC CTTTTCTGTC
151 AGTCCACTTC ACCAAGCCTG CCCTTGGACA AGGACCCGAT GCCCAACCCC
201 AGGCCTGGCA AGCCCTCGGC CCCTTCCTTG GCCCTTGGCC CATCCCCAGG
251 AGCCTCGCCC AGCTGGAGGG CTGCACCCAA AGCCTCAGAC CTGCTGGGGG
301 CCCGGGGCCC AGGGGGAACC TTCCAGGGCC GAGATCTTCG AGGCGGGGCC
351 CATGCCTCCT CTTCTTCCTT GAACCCCATG CCACCATCGC AGCTGCAGCT
401 GCCCACTG TGCTTAGTCA TGGTGGCACC CTCCGGGGCA CGGCTGGGCC
451 CCTTGCCCCA CTTACAGGCA CTCCTCCAGG ACAGGCCACA TTTTCATGCAC
501 CAGCTCTCAA CGGTGGATGC CCACGCCCCG ACCCCTGTGC TGCAGGTGCA
551 CCCCCTGGAG AGCCAGCCA TGATCAGCCT CACACCACCC ACCACCGCCA
601 CTGGGGTCTT CTCCCTCAAG GCCCGGCCTG GCCTCCCACC TGGGATCAAC
651 GTGGCCAGCC TGAATGGGT GTCCAGGGAG CCGGCACTGC TCTGCACCTT
701 CCCAAATCCC AGTGCACCCA GGAAGGACAG CACCCTTTCG GCTGTGCCCC
751 AGAGCTCCTA CCCACTGCTG GCAAATGGTG TCTGCAAGTG GCCCGGATGT
801 GAGAAGGTCT TCGAAGAGCC AGAGGACTTC CTCAAGCACT GCCAGGCGGA
851 CCATCTTCTG GATGAGAAGG GCAGGGCACA ATGTCTCCTC CAGAGAGAGA
901 TGGTACAGTC TCTGGAGCAG CAGCTGGTGC TGGAGAAGGA GAAGCTGAGT
951 GCCATGCAGG CCCACCTGGC TGGGAAAATG GCACTGACCA AGGCTTCATC
1001 TGTGGCATCA TCCGACAAGG GCTCCTGCTG CATCGTAGCT GCTGGCAGCC
1051 AAGGCCCTGT CGTCCCAGCC TGGTCTGGCC CCCGGGAGGC CCTTGACAGC
1101 CTGTTTGCTG TCCGGAGGCA CCTGTGGGGT AGCCATGGAA ACAGCACATT
1151 CCCAGAGTTC CTCCACAACA TGGACTACTT CAAGTTCAC AACATGCGAC
1201 CCCCTTTCAC CTACGCCACG CTCATCCGCT GGGCCATCCT GGAGGCTCCA
1251 GAGAAGCAGC GGACACTCAA TGAGATCTAC CACTGGTTCA CACGCATGTT
1301 TGCCTTCTTC AGAAACCATC CTGCCACCTG GAAGAACGCC ATCCGCCACA
1351 ACCTGAGTCT GCACAAGTGC TTTGTGCGGG TGGAGAGCGA GAAGGGGGCT
1401 GTGTGGACCG TGGATGAGCT GGAGTTCCGC AAGAAACGGA GCCAGAGGCC
1451 CAGCAGGTGT TCCAACCTTA CACCTGGCCC CTGACCTCAA GATCAAGGAA
1501 AGGAGGATGG ACGAACAGGG GCCAACTGG TGGGAGGCAG AGGTGGTGGG
1551 GGCAGGGATG ATAGGCCCTG GATGTGCCCA CAGGGACCAA GAAGTGAGGT
1601 TTCCACTGTC TTGCCTGCCA GGGCCCCTGT TCCCCGCTG GCAGCCACCC
1651 CCTCCCCCAT CATATCCTTT GCCCCAAGGC TGCTCAGAGG GGCCCCGGTC
1701 CTGGCCCCAG CCCCCACCTC CGCCCCAGAC ACACCCCCCA GTCGAGCCCT
1751 GCAGCCAAAC AGAGCCTTCA CAACCAGCCA CACAGAGCCT GCCTCAGCTG
1801 CTCGCACAGA TTAATTTCAG GCTGGAAAAG TCACACAGAC ACACAAAATG
1851 TCACAATCCT GTCCCTCAC

```

Figure 3

09697340-102400

## HUMAN FKH<sup>st</sup> PROTEIN SEQUENCE

```
1  MPNPRPGKPS APSLALGPSP GASPSWRAAP KASDLLGARG PGGTFQGRDL
51  RGGAHASSSS LNPMPPSQLQ LPTLPLVMVA PSGARLGPLP HLQALLQDRP
101 HFMHQLSTVD AHARTPVLQV HPLESPAMIS LTPPTTATGV FSLKARPGLP
151 PGINVASLEW VSREPALLCT FPNPSAPRKD STLSAVPQSS YPLLANGVCK
201 WPGCEKVFEE PEDFLKHCQA DHLLDEKGRA QCLLQREMVO SLEQQLVLEK
251 EKLSAMQAH LAGKMALTKAS SVASSDKGSC CIVAAGSQGP VVPAWSGPRE
301 APDSLFAVRR HLWGSHGNST FPEFLHNMDY FKFNMRPPF TYATLIRWAI
351 LEAPEKQRTL NEIYHWFTRM FAFFRNHPAT WKNAIRHNLS LHKCFVRVES
401 EKGAVWTVDE LEFRKRSQR PSRCSNPTPG P*
```

Figure 4

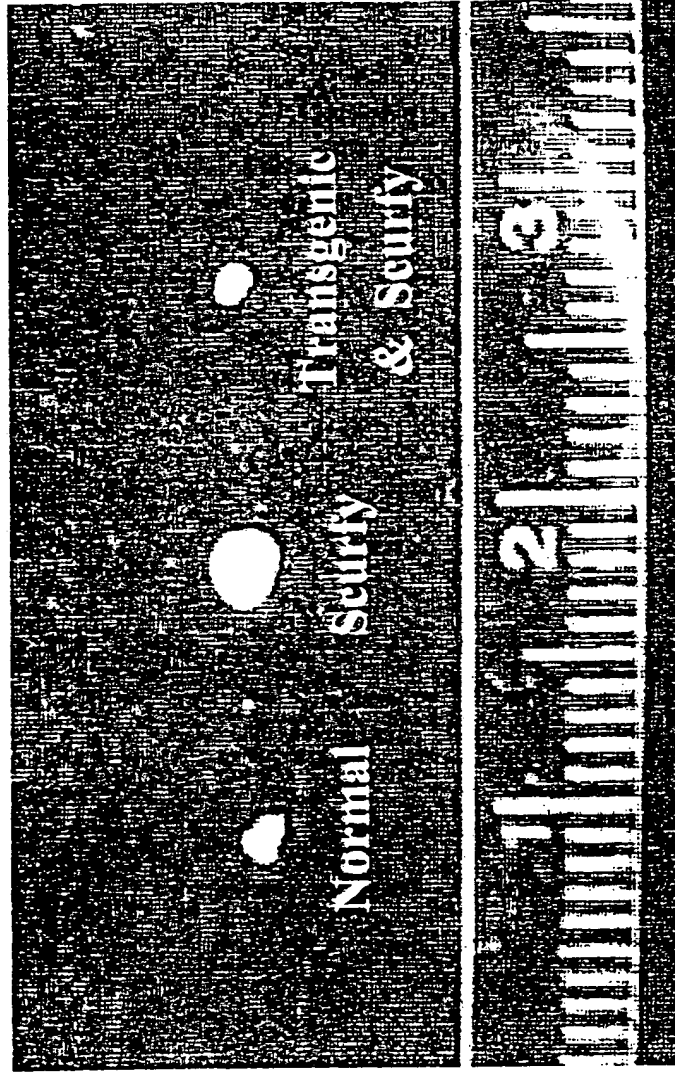
004207"04E26960

# Vector for generation of FKH<sup>sf</sup> Transgenic mice



Figure 5

# FKHsf Transgene corrects the defect in scurfy animals



BEST AVAILABLE COPY

Figure 6

# FKHsf tg mice have reduce lymph node cells compared to normal cells

Cell number	Mouse genotype	
	Normal	Scurfy Transgenic
Cells / LN	0.92	1.97 0.29
Cells / Thymus	0.76	0.54 0.76

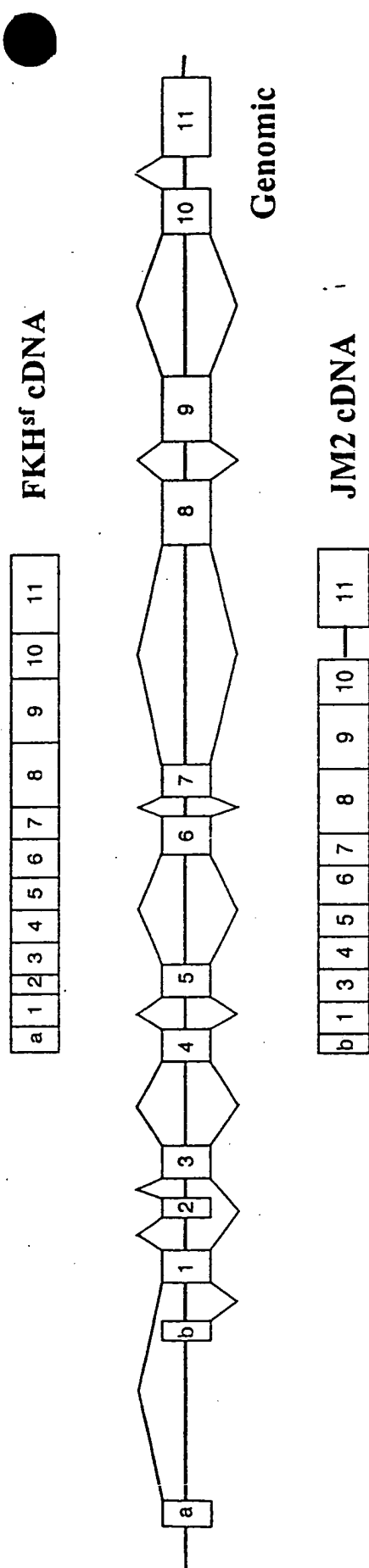
Figure 7

# **FKHsf transgenic mice respond poorly to in vitro stimulation**

	Mouse genotype	
	Normal	Scurfy Transgenic
Proliferation		
No stimulation	778	23488
Anti-CD3+Anti-CD28	22932	225981
		9106

Figure 8





**Comparison of FKH<sup>sf</sup> and JM2 cDNAs.** Exon/intron structure is shown (Genomic) as open rectangles (exons) joined by heavy horizontal lines (introns). Coding exons are numbered 1-11 as determined by sequence analysis of FKH<sup>sf</sup> cDNA; non-coding 5' exons are labelled *a* and *b*. The FKH<sup>sf</sup>-specific and JM2-specific splicing patterns and resulting cDNAs are indicated above and below the genomic structure, respectively.

**Figure 9**

		<u>Human FKHS<sup>f</sup></u>	
<i>N-terminal</i>	<i>ZNF</i>	<i>Mid</i>	<i>Forkhead</i>
83.4%	95.8%	82.8%	96.4%
		<u>Mouse Fkh<sup>sf</sup></u>	

Human and mouse FKHS<sup>f</sup> proteins are highly conserved.

Figure 10